REMARKS

By the present preliminary amendment, claims 1-7 have been canceled without prejudice. Applicants maintain the right to prosecute the canceled claims in any related application claiming the benefit of priority of the subject application. New claims 8-25 have been added. Accordingly, upon entry of the preliminary amendment, claims 8-25 are pending.

Support for new claims 8-25 can be found throughout the specification. In particular, claim 8, steps (a) to (d) are supported, for example, at page 6, line 7, to page 8, line 2; and steps (e) to (f) are supported, for example, at page 18, lines 16-28. Claim 9 is supported, for example, at page 10, line 25, to page 11, line 3. Claims 10 and 18, steps (a) to (f), are supported, for example, at page 9, line 12, to page 10, line 13. Claim 10, steps (g) and (h), are supported, for example, at page 7, line 8, to page 8, line 21. Claim 18, steps (g) to (m), are supported, for example, at page 6, line 20 to page 7, line 3; at page 14, line 26, to page 16, line 7; and at page 18, lines 5-28. Claims 11 and 19 are supported, for example, at page 12, line 24, to page 13, line 4.

The expression $r \geqq L+1$, is inherently supported in the specification. The target mRNA or its precursor is a single chain. Thus, the formation of a double–stranded region (as a result of internal self-hybridization within the single chain) results in a loop consisting of unpaired bases existing between the two strands which make up the double-stranded region (page 17, lines 11-18, and Figure 2). The specification discloses that L is the minimum number of bases in a loop (page 12, line 2-3 from the bottom) and because the loop consists of unpaired bases, as discussed above, does not include bases in the double-stranded region. L varies from 3-10 (page 13, line 2).

On the other hand, r is the "number of bases between 2 regions +1" (page 12, line 1 from the bottom), which means the "number of bases in a loop +1" from the above fact that bases present between the two strands which make up the double-stranded region make the loop. r is an integer of 1 or more (page 13, lines 2-3), because r is a distance between one of double strand and other strand (page 12, line 2 from the bottom to page 13, line 3).

In examining the possibility of forming a complementary double-stranded region, the minimum value of "r" may be set e.g. at 4 to 11, preferably 5 to 7 (page 14, lines 20-21) hence

corresponding to the value of L, and a region located with a distance of less than (minimum) "r" between regions is excluded from the calculation (page 15, lines 1-4). Therefore, based upon the relationship between L and r disclosed in the specification, the minimum value of r is equal to "L +1" ($r \geqq L+1$).

Claims 12 and 20 are supported for example, at page 11, lines 4-13. Claims 13-15 and 21 to 23 are supported, for example, at page 13, lines 2-4. Claims 16, 17, 24 and 25 are supported, for example, as set forth above for claims 12-15 and 20-23. Thus, as new claims 8-28 are supported by the specification, no new matter has been added. Accordingly, Applicants respectfully request entry of the new claims.

THE INVENTION

The present invention, in various embodiments, is directed to identifying antisense targets in an mRNA or its precursor by identifying which sequences are likely to form intramolecular double strands and which sequences are less likely to form intramolecular double strands. A method includes comparing complementarity of sequence regions with all other regions in the given sequence and assigning a numerical value to the region based upon their probability of forming a complementary pair. A relatively low score indicates a lower probability of forming a double strand pair than a higher score.

The calculated score depends on the distance between sequence regions for the paired nucleotides and the bond energy ΔG between nucleotides of the pair. The score decreases as the distance between the two regions increase and as the bond energy decreases. The score increases as the distance between the two regions decrease and as the bond energy increases. Thus, sequence regions having lowest bond energy due to sequence noncomplementarity and the greatest distance between them will have the lowest scores and will be better targets for antisense than sequence regions having the highest scores due to greater bond energy and a shorter distance between the regions. For example, in one embodiment, distance between sequence regions and bond energy are reflected in calculating the numerical value for forming a double strand sequence in the formula, $((L+1)/r)^F \exp(|\Delta G|/RT)$.

Although art has been cited during prosecution of the subject application that allegedly describes Applicants' invention methods, the cited art method is different from the invention methods in several respects. First, the cited art method requires a prediction of overall secondary structure in order to identify single stranded regions, based upon a calculation of the lowest free energy of the overall structure. However, in contrast to the cited art method, the invention methods do not require a determination of secondary structure(s) nor a calculation of the overall stabilized energy of the sequence in order to identify possible antisense targets. Rather, the methods of the invention are performed iteratively by comparing sequence regions to identify the lowest scoring regions (which as discussed depends on distance and free energy). That is, each and every sequence region within a particular sequence is compared to all other sequence regions in a step wise fashion thereby identifying region(s) having lowest relative score(s). In this way, an antisense target is identified without determining the secondary structure of the entire sequence and without having to calculate overall stabilized energy of the sequence.

Second, in stark contrast to the invention methods, the cited art method does not include distance between two sequence regions as a parameter that affects the probability of forming an intramolecular double strand. Thus, in the methods of the invention, distance influences the probability of forming a double strand region, which affects the score thereby affecting the target region selected for antisense, whereas the cited art method does not account for distance between two sequence regions in identifying antisense target sites.

Third, as made of record during prosecution of the subject application, the cited art method for predicting RNA secondary structure is also limited to a 750 base fragment (see, for example, Jaeger *et al.*, Methods Enzymol. 183:281 (1990), page 287, lines 17-19, previously cited in form PTO-892). In contrast, the invention methods are not so limited to 750 base pairs. Accordingly, for the aforementioned and other reasons, the methods of the invention are clearly distinct from the cited art method.

Applicants submit that the claims are in condition for examination, which action is respectfully requested. If the Examiner would like to discuss any issues relating to the application, she is encouraged to contact the undersigned. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

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